TARGETING TOPOISOMERASE(S) FOR ANTI LEISHMANIAL CHEMOTHERAPY AND UNDERSTANDING THE MECHANISM OF TOPOISOMERASE RELATED DNA DAMAGE REPAIR MACHINERIES IN *Leishmania donovani*

Abstract

The first segment of the investigation establishes three molecules, (i) voacamine, an indole alkaloid isolated from *Tabernaemontana coronaria,* (ii) 'Compound 2', a novel bisbenzylisoquinoline alkaloid isolated from *Thalictrum foliolosum*, and (iii) JVPH3, an isobenzofuranone derivative, as potential drug candidates against visceral leishmaniasis caused by *Leishmania donovani.* The study elucidates that voacamine and 'Compound 2' exert their antileishmanial activities by targeting type 1B topoisomerase of *L. donovani* (LdTop1B) whereas JVPH3 targets type 2 topoisomerase (LdTop2). However, despite targeting LdTop1B, voacamine stabilizes LdTop1cc but 'Compound 2' abrogates LdTop1cc formation and acts as catalytic inhibitor. JVPH3 hampers the catalytic activity of LdTop2 and does not stabilize LdTop2cc.

When subjected to LdTop1B poisons, parasites must activate some machinery to repair the stabilized 'topoisomerase 1 induced cleavage complex' (LdTop1cc) being generated. Accumulation of this LdTop1cc is the prime reason for cytotoxicity. The second and third segments of the study unravel that *L. donovani* possesses two distinct pathways primarily led by TDP1 and MRE11 dedicated to LdTop1cc repair in this protozoa. Both of these proteins in *L. donovani* hydrolyze the 3′-phosphotyrosyl adducts generated by topoisomerase 1 poisons. This is indispensible to release LdTop1B from the trapped LdTop1cc so that the cells can survive. The study also establishes the potential crosstalk between TDP1 and MRE11 in response to topoisomerase poisoning. TDP1 is more critical than MRE11 for the parasite to fight against Top1cc induced cell death. Absence of MRE11 can be replenished by enhancing the TDP1 level as far as LdTop1B associated cytotoxicity is concerned. However, the reverse case does not hold.

Finally, when drug resistance has become a significant problem, the current study also establishes that TDP1 is related to drug resistance in *L. donovani.* Drug resistant field isolates of *L. donovani* downregulates their endogeneous TDP1 level eventually giving rise to a new concept that topoisomerases of different resistant strains of *L. donovani* may be targeted to kill them. Cumulatively, the investigation demonstrates the efficacies of three molecules as potential antileishmanial agents which act by targeting the topoisomerases of the parasite and highlights the roles of two molecular pathways associated with Top1cc repair in *Leishmania donovani.*

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